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Characterization of MMP-3 from Rheumatoid Arthritis Patients and the Production of Rabbit Polyclonal Anti-MMP-3 Antibody

Sulistiana Prabowo[a]*, Sartono Sartono[b], Irmawati Dikman[c], Fitri Handajani[d], Aulanni'am Aulanni'am[e]

[a,b,c,d]*Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia.*

[e]*Faculty of Veterinery Medicine, Brawijaya University, Malang, Indonesia.*

[a]*Email: sulis_prabowo@yahoo.co.id*

Abstract

Rheumatoid arthritis (RA) is a chronic, systemic disorder characterised by inflammation of the joint synovial membrane associated with cytokine release resulted in progressive joint destruction. RA pathogenesis was thought to be initiated by bone resorption by osteoclasts, immune system activation and matrix metalloproteinase (MMP) production. Serum MMP-3 levels at the onset of disease is predictive of joint damage progression and thought to play an important role in joint destruction in RA. This study aimed to determine the presence of elevated serum MMP-3 in RA patients and to produce custom made patient derived anti-MMP-3 antibody for diagnostic and prognostic purposes. We found significant increase of serum MMP-3 in RA patients participated in this study and able to produce anti-MMP-3 antibody from their serum. The success of MMP-3 antibody production from RA patients in this study is novel and will be beneficial for future diagnostic and prognostic purposes for patients from similar geographical area. Currently none of the MMP-3 antibodies which are available commercially were produced in the Asian countries which raised questions about their specificity for the local use.

Keywords: MMP-3; rheumatoid arthritis; antibody; rabbit.

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* Corresponding author.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory immune disorder that primarily affects the joints characterised with inflammation of synovial membranes and the release of inflammatory cytokines resulted in progressive joint destruction[1-4]. The progressive long term effect of joint destruction in RA leads to functional impairment, deterioration in quality of life, and severe disability [1, 3]. The aetiology of RA has not been clearly defined but previous investigators believe that RA pathogenesis was initiated by bone resorption in the joint due to the action of osteoclasts [5] followed by immune system activation and matrix metalloproteinase (MMP) production. Early features of rheumatoid joints is characterised by inflammatory cell infiltration associated with neovascularization and proliferation of synovial tissue. The areas of overgrown synovial tissue eventually became the major source of pro-inflammatory cytokines and MMPs production [4].

MMPs are large group of zinc dependent proteases which have the ability to degrade component of extracellular matrix such as elastin, collagen, gelatine, and casein [6]. Serum levels of the MMPs including MMP-1, -3, -8, -9 have been shown to be associated with disease activity in early RA [7, 8]. MMP-3 (stromelysin-1) is one of MMP family member that produced in the joints and it is a proteinase with wide range of substrate specificity. MMP-3 activity result in degradation of aggregan core protein, cartilage link protein, fibronectin, and collagen type III, IV, VII, IX, X and XI. MMP-3 can aggregate inflammation by activating pro-MMP-1, pro-MMP-7, pro-MMP-8, pro-MMP-9 and pro-MMP-13. MMP-3 is secreted by fibroblasts, chondrocytes and synovial cells and is considered the most important proteinase that responsible for cartilage degradation [4, 6]. MMP-3 levels has been shown to accurately predicted ongoing bonedestruction during Biological Agent treatment[2] and serum pro-MMP-3 levels at the onset of disease are also predictive of joint damage progression and thought to play an important role in joint destruction in RA [7, 8].

MMP-3 is locally produced in the inflamed joint and then released to the circulation [9]. Systemic MMP-3 levels are a reflection of local synthesis and it was suggested that serum MMP-3 level is correlated with MMP-3 produced by synovium and reflected the activity of RA [10, 11]. Previous investigators have shown that serum MMP-3 can be used as a systemic marker of local joint inflammation and/or destruction [12] and that serum concentration of MMP-3 in early stage of RA can be used as a marker for predicting the level of future progressive bone damage [4, 13]. Furthermore treating RA with the aim to target both normalisation of MMP-3 as well as lowering the disease activity score to below 2.6 seems to yield better effects than treating RA with the aim of only either one of them [14].

As mentioned above previous studies have shown that serum MMP-3 level resembles the local joint synthesis and that arthritis progression is associated with increased level of serum MMP-3. The degree of increased level of serum MMP-3 can potentially be used as a reliable marker to determine the occurrence of disease activity and rate of future progressive joint destruction in RA and thus the prognosis of the disease [6, 15, 16]. The level of expression of serum MMP-3 can be easily measured from blood using venous puncture which is reasonably simple and non-invasive thus it is a favourable diagnostic tool.

Caution is needed when using MMP-3 as an RA marker because of the presence of sex difference in the normal

range of MMP-3. The cut off value of serum immuno assay is twice as high for men as for women[17]. Furthermore serum MMP-3 level in non RA individuals is influenced by various treatments including glucocorticoids [18]. Glucocorticoid teatment in non RA individuals markedly increased the level of serum MMP-3 although the source of MMP-3 and the mechanism involved are unclear. [19-21]. In individuals with RA however, serum MMP-3 level were markedly increase regardless of being treated with/without glucocorticoids. Therefore if serum MMP-3 is to be used as a diagnostic tool for RA, the exclusion criteria for baseline MMP-3 in controls should include the past and current use of glucocorticoid therapy[18].

To characterize the presence of elevated serum MMP-3 in RA patients and to determine the occurrence of significant increase level of serum MMP-3 in RA patients in comparison to healthy individuals without RA the use of specific identification measures is pertinent. The MMP-3 specificity is crucial and the production of custom made antibody from RA sufferers with the same geographical location and environment is likely to be more accurate determinant factor and can minimise potential external factor variabilities. Due to the importance of finding an accurate M MP-3 marker to assist RA diagnosis and prognosis this study aimed to custom develop an MMP-3 antibody sourced from RA sufferer's MMP-3 over production. If successful it is potentially can be used as a reliable marker to detect MMP-3 expression in RA patients from a specific geographical area. The antibody specificity will reduce or eliminate potential variabilities that could happen when using commercially available antibodies. Before the development of the anti-MMP-3 antibody the presence of significant elevation of serum MMP-3 in RA patients was firstly determined. The serum MMP-3 obtained from RA patients was characterized, isolated and then used to induce the production of anti-MMP-3 polyclonal in rabbits.

2. Materials & Methods

Five patients diagnosed with an established RA according to 1987 American College of Rheumatology (Arnett and his colleagues 1988) from Dr Soetomo Public Hospital, Surabaya, Indonesia (F; 40-55 yo) were recruited for this study. Controls were obtained from healthy individuals showed no sign of RA (M; 25-35 yo) and none of them were being treated with corticosteroids prior or at the time of their involvement in the study. Five ml blood were taken from each participants and centrifuged straight away at 3500 rpm for 20 minutes at room temperature to obtain the serum. The serum was stored in -80^{O}C freezer until being used. Written and informed consent was obtained from all participants and the study was approved by the ethics committee at Dr Soetomo Public Hospital, Surabaya, Indonesia.

2.1. Determination of MMP-3 level using indirect ELISA and SDS-PAGE methods

All experiments were done in triplicates. Serum from RA patients and controls were thawed to room temperature. Using ELISA kit (Sigma-Aldrich, St. Louis, MO, USA) samples of 100 µL in volume were inserted in each microplate well and incubated in refrigerator at 4^{O}C for 24 hr. They were then washed in 0,05% PBS-Tween for 3 minutes and repeated 3 times and blocked with 100 µL blocking buffer PBS-BSA 1% for 2 hr at room temperature, washed again with 0,05% PBS-Tween for 3 minutes and repeated 4 times. Primary antibody reaction was using 100 µL polyclonal anti human anti MMP-3 (Sigma-Aldrich) diluted 1:40 in blocking buffer and incubated for 2 hr at room temperature. After washing with 0,05% PBS-Tween (PBS-T) for

3 minutes and repeated 4 times 100 µL secondary antibody (IgG Biotin Labelled) was applied at the dilution of 1:2500 in PBS-T and incubated at room temperature for 2hr. After washing with PBS-T for 3 minutes and repeated 3 times 100 µL SA-HRP substrate was added in each well and incubated for 30 min at room temperature in the dark. Once the colour of the controls turned yellow the reaction was stopped by adding 100µl 1M HCL (Stop Reaction). The reading was done twice using ELISA reader at 450 nm wavelength. Negative controls were treated exactly the same without adding the antigen (sera RA patient or control).

SDS-PAGE was used to determine the molecular weight of protein bands in the samples including MMP-3. Briefly 35 µL isolate serum protein were fed into each of the wells of SDS-PAGE gel next to a molecular weight marker and electrophoresed for 1.5 h at 130 V and 30 mA. The gel were then stained with*coomassie blue* and put on a shaker for 30 min, destained repeatedly until the solution was cleared and the blue protein bands can be observed clearly. The possible presence of MMP-3 can be identified based on the molecular weight marker of the band by Western blot technique using MMP3 monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA)

2.2. To determine the specificity of MMP-3 using Western Blotting methods

Western blot method was used to determine whether the protein that has a similar size with MMP-3 observed in SDS-PAGE gel was actually MMP-3.

The protein in SDS-PAGE gel were transfered to Nitrocellulose (NC) membraneand blocked with 3% BSA in 20 mM Tris-NaCl pH 7,5 and 150 mM NaCl for 1 hr. It was then incubated in Tris-Cl buffer containing 1% BSA with monoclonalanti-MMP-3 as the primary antibody overnight (Sigma-Aldrich, St. Louis, MO, USA). The membrane was then washed twice in Tris-Cl buffer containing 0,05% Tween 20 for 10 min. The membrane was then incubated in secondary antibody anti human anti IgG alkaline phosphatase conjugated at 1:2500 dilution (Sigma-Aldrich, St. Louis, MO, USA) for 1 hour, It was then washed again twice and stained with Western bluesubstrate to reveal the MMP-3 bands.

For collecting Human MMP3 from the sera using unstained Native PAGE, gel was then cut along the confirmed MMP-3 band by Western blot. Each piece was inserted in the dialysis bag containing 1-2 ml of 0.05M phosphate buffer and electroeluted overnight at $4^{O}C$ (220V, 20 mA).

The gel was stained with coomassie blue to confirm that the protein has been electroeluted and there was no MMP-3 band left. The electroeluted protein was then precipitated in cold absolute ethanol and the pellet was collected. The MMP-3 pellet will then be used as immunogen for inducing MMP3 polyclonal antibody production.

2.3. Immunisation and measurement of anti-MMP-3 polyclonal antibody in rabbits

The 54 kDa MMP-3 protein (200 µL isolate protein in 200 mlCFA) that has been characterized from the participants was injected to 8 rabbits to generate anti-MMP-3 polyclonal antibody. The protocol of MMP-3 immunization and harvest of MMP-3 antibody is showed in the below (Table 1).

Table 1: MMP-3 immunisation and MMP-3 antibody harvest in rabbit

Day	Activity	Treatment
0	*Bleeding (pre*-treatment)	
1	First Immunisation	200µl MMP-3 isolate + 200 µLCFA (Complete Freund Adjuvant)
28	Second Immunisation *(booster* 1)	200µl MMP-3 isolate + 200µL CFA
38	*Bleeding* 1	
45	*Bleeding* 2	
52	*Bleeding* 3	
59	*Bleeding* 4	
66	*Bleeding* 5	
69	Third Immunisation (*booster* 2)	200µl MMP-3 isolate + 200µL CFA
81	*Bleeding* 6	
88	*Bleeding* 7	
96	*Bleeding* 8	
103	*Bleeding* 9	
110	*Bleeding* 10	

Blood was collected and the serum level of polyclonal MMP-3 antibody was determined using indirect ELISA methods as previously described.

Antigen (MMP-3) at a concentration of approximately 100 µg/ml in 100 µL Carbonate-Bicarbonate buffer (Coating buffer) was placed in eachmicroplateand incubated at 4º C for 24 h. They were then washed 4 times in PBS-T for 3 min each and blocked with 100 µL Blocking buffer and incubated at room temperature for 2 h. After washing 100 µL primary antibody (polyclonal anti-MMP-3 from rabbit) was added at a concentration of 1:40 and incubated for 2 h at room temperature. After another washing 100 µL secondary antibody was added (*Anti Rabbit IgGBiotin Labelled*) at a concentration of 1/2500 and incubated for 2 h at room temperature. After washing 100 µL SA-HRP substrate was added and incubated for 30 min at room temperature in the dark. Once the control colour turned yellow the reaction was stopped by adding 100 µl 1M HCL (Stop Reaction). Titre was measured using ELISA reader at the wavelength of 450 nm. Negative control was without antigen.

2.4. Characterisation of MMP-3 produced by rabbits using DOT BLOT and Western blot

Dot blot is a method used to test the presence of an antigen. Protein to be tested was put on a membrane and measured semi quantitatively by colour intensity using Corel Photopaint 12 program. Briefly MMP-3 isolate (50 µl) was placed on the membrane and incubated for 30 min, blocked with PBS Skim 1% for 1 h and washed with

PBS-T (3x3 min). Primary antibody was added (polyclonal anti-MMP-3 from rabbit) and incubated for 2 h on a shaker, washed and incubated in secondary antibody Anti Rabbit IgG Alkaline Phospatase (1:2500) for 1 h. The membrane was then washed in PBS-T (3x3 min) then incubated in Western Blue Substrate Solution for 30 min. The reaction was stopped with H_2O and the presence of colour and its intensity was observed and compared.

Serumwas electrophoresed using SDS-PAGE as previously explained. The bands that present on the gel were then characterised to determine the presence of MMP-3 using Western blot methods and polyclonal anti-MMP-3 antibody as previously explained.

2.5. Statistics

The level of serum MMP-3 measured by indirect ELISA from RA patients and controls was compared using Mann Whitney test.

3. Results

3.1. Serum MMP-3 concentration obtained from participants

MMP-3 concentration from serum obtained from all participants was measured as the average of two measurements using indirect ELISA methods, listed below on Table 2.

Using Mann Whitney test significant increase was found in RA patients serum MMP-3 level compare to controls ($p = 0.020$).

Table 2: Serum MMP-3 concentration from all participants

Sample	Absorbance 1	Absorbance 2	Average	Concentration (ppm)
Control 1	0,386	0,402	0,394	4,000
Control 2	0,403	0,405	0,404	5,667
Control 3	0,396	0,414	0,405	5,833
RA 1	0,485	0,478	0,482	18,583
RA 2	0,478	0,486	0,482	15,917
RA 3	0,48	0,451	0,466	18,667
RA 4	0,496	0,52	0,508	23,000
RA 5	0,495	0,489	0,492	20,333
RA 6	0,496	0,486	0,491	20,167

3.2. MMP-3 characterisation using Western blot

MMP-3 from serum obtained from controls and RA patients was characterised by Western blot showing MMP-3

bands (54 kDa). These were then be eluted and isolated (Figure 1).

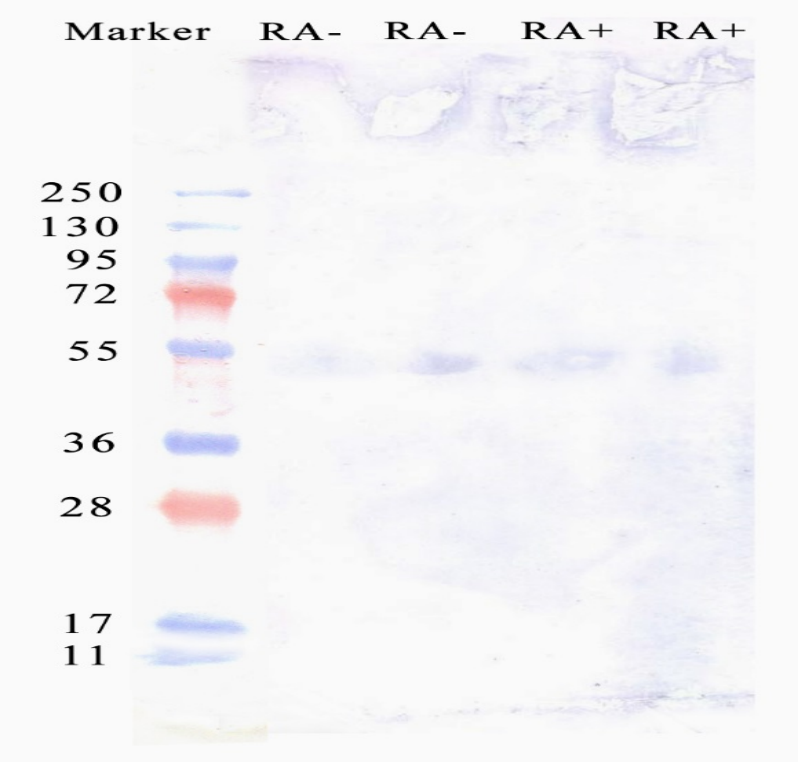

Figure 1: MMP-3 bands (54 kDa) from serum obtained from controls and RA patients

3.3. MMP-3 measurements in immunised rabbits

The concentrations of MMP-3 from rabbits were measured after immunisation as shown on table 3.

Table 3: MMP-3 concentration from rabbits after immunisation.

Bleeding no.	Absorbance			Concentration after dilution 20x (ppm)	Concentration before dilution 20x (ppm)
	1	2	Average		
0	0,374	0,377	0,3755	0,9167	18,33
1	0,419	0,414	0,4165	7,75	155,00
2	0,427	0,421	0,424	9	180,00
3	0,440	0,444	0,442	12	240,00
4	0,408	0,436	0,422	8,6667	173,33
5	0,412	0,420	0,416	7,6667	153,33
6	0,435	0,436	0,4355	10,9167	218,33
7	0,444	0,446	0,445	12,5	250,00
8	0,456	0,455	0,4555	14,25	285,00
9	0,422	0,413	0,4175	7,9167	158,33
10	0,403	0,411	0,407	6,1667	123,33

3.4. Characterisation using DOT BLOT

DOT BLOT experiment showed that all post immunisation bleeding were positive against MMP-3 while pre immunisation bleeding was negative confirming that the antibody detected in all post immunisation bleeding was polyclonal anti-MMP-3 antibody (Figure 2).

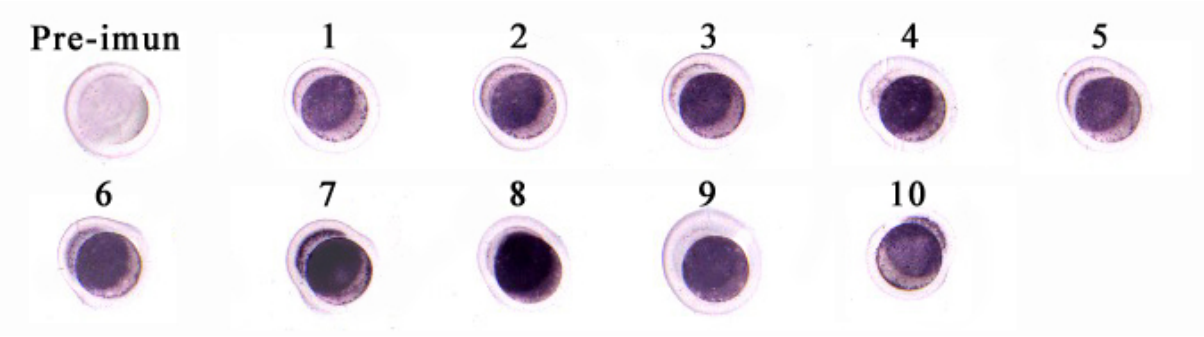

Figure 2: Dot blot experiment showed positive reaction from all bleeding 1 – 10 post immunisation and negative reaction from pre immunisation bleeding confirming the presence of polyclonal anti-MMP-3 antibody.

3.5. Identification of serum rabbit polyclonal MMP-3 antibody using Western blot

Identification of polyclonal anti-MMP-3 antibody from the rabbit serum using Western blot (Figure 3) showed that positive MMP-3 band of controls and RA patients were the same as using monoclonal anti-MMP-3 antibody. It confirmed that this antibody was polyclonal anti-MMP-3 antibody.

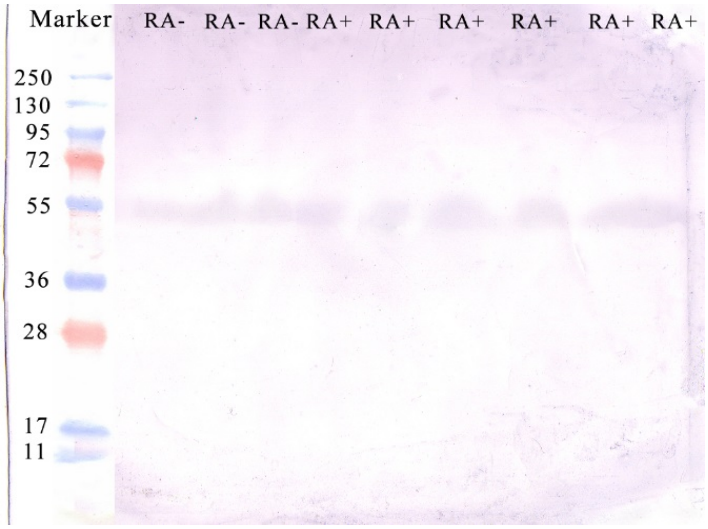

Figure 3: Western blot experiment showed positive bands in the gels that matched the molecular weight size of MMP-3 using monoclonal anti-MMP-3 antibody

4. Discussion

MMP-3 has been shown to cleave a series of ECM proteins and is considered as an important protease in the

progression of joint damage in RA patients [22, 23]. Previous studies have indicated the prime importance of elevated synovial MMP-3 in RA which is positively correlated with synovitis assessed by comprehensive histological and inflammatory cell analysis [16]. Serum MMP-3 was also shown to be significantly correlated with synovial MMP-3 and synovitis level and had the ability to distinguish high grade from low grade synovitis in RA [16]. Supporting previous studies in this study we showed the presence of significant elevation of serum MMP-3 by indirect ELISA in patients with active progressive RA in comparison to controls which has no RA using Mann Whitney test ($p = 0.020$). The difference could potentially be greater as we used male controls which are supposed to have significantly higher level of baseline serum MMP-3 in comparison to females [17]. At least we have the confidence that despite using male controls we can show 4 fold increase in the level of serum MMP-3 from our female RA patients. Such MMP-3 has molecular weight 54 kDa, which is the whole MMP-3. Up to date detection of MMP-3 levels is limited by the availability of commercial assays, which potentially have unknown variabilities. We believe that custom made antibody sourced from individuals with similar geographical and disease condition could eliminate some of those unknown variabilities. As far as we are aware MMP-3 antibody from human RA source was never been developed in Indonesia to date and this study is the first attempt to develop this antibody in that geographical area. It potentially will be able to be used to more specifically detect MMP-3 in RA patients from similar disease background, race and area. In this study we have successfully developed rabbit polyclonal anti-MMP-3 antibody from the RA patients that showed MMP-3 specificity by dot blot, Western blot and ELISA methods. Previous investigators have developed monoclonal active MMP-3 in mice and found its positive expression in cultured synovial membrane and human cartilage explant supernatant [15]. They also discovered that active MMP-3 expression was significantly decreased after anti-TNF-α treatment, which could indicate that active MMP-3 could be used as a predictor of the treatment efficiency at the level of anti-inflammatory effects. The levels of expression however did not correlate to or predict changes in disease activity scores. Furthermore, active MMP-3 significantly correlated with CRP and ESR levels, which indicated that active MMP-3 plays important role and responsible for inflammation. In other studies serum whole MMP-3 was found as an independent predictor of structural damage progression in patients with ankylosing spondylitis [24] but no correlation was found in in active MMP-3 expression in RA *in vitro* cultured synovial membrane and human cartilage explant which indicate active MMP-3 behaves different than the classical inflammatory markers [15]. Previous finding has reported the limitations in relation to the detection of active MMP-3 in human serum. Active MMP-3 is known to bind to carrier proteins like TIMP and α-2 macroglobulin which are abundant in biological fluid and shield them in the subunits [25-27]. This could result in the difficulties in identifying the real expression of active MMP-3 and skew the levels in serum towards lower levels. The expression of active MMP-3 was shown in cultured cells and its usefulness as RA disease marker in human is still not confirmed [15]. Many investigators have found the correlation between serum MMP-3 expression and the disease activity and progression in human RA which was also shown in this study [3, 6, 8, 16, 24, 28]. Based on this consideration we believe that whole MMP-3 is a better alternative for RA marker. We believe that this study supported previous findings about the importance of serum MMP-3 in RA and could potentially be used for diagnostic purposes. Moreover we also showed in this study the ability to produce polyclonal MMP-3 antibody from RA patients in rabbits which could be very beneficial for use for community with similar geographical areas and background. This paper has limitation where the number of samples was relatively small and all participants were recruited from the same hospital.

Further studies using larger number of samples recruited from different hospitals and/or different cities/countries is recommended to determine whether the similar upregulation of MMP-3 expression in RA can be found. Furthermore the properties of MMP-3 antibody production obtained from RA sufferers different geographical sites can be compared to determine the differences/similarities of the antibody produced based on geographical location. Recommendation then can be made on the most suitable antibody that can be used to identify/diagnose RA disease in a particular group of RA sufferers.

5. Conclusion

In this study we found significant increase of serum MMP-3 in our group of RA patients. The production of anti-MMP-3 antibody from their serum will be beneficial for future diagnostic and prognostic purposes for patients from similar geographical area. Currently none of the MMP-3 antibodies which are available commercially were produced in the Asian countries which raised questions about their specificity for the local use.

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References

[1]. Mamehara A, Sugimoto T, Sugiyama D, Morinobu S, Tsuji G, Kawano S, et al. Serum matrix metalloproteinase-3 as predictor of joint destruction in rheumatoid arthritis, treated with non-biological disease modifying anti-rheumatic drugs. Kobe J Med Sci.56(3):E98-107.2010.

[2]. Kaito T, Ohshima S, Fujiwara H, Makino T, Yonenobu K. Predictors for the progression of cervical lesion in rheumatoid arthritis under the treatment of biological agents. Spine (Phila Pa 1976). 38(26):2258-63. 2013.

[3]. Hiura K, Iwaki-Egawa S, Kawashima T, Fujisawa S, Takeda T, Komori H, et al. The diagnostic utility of matrix metalloproteinase-3 and high-sensitivity C-reactive protein for predicting rheumatoid arthritis in anti-cyclic citrullinated peptide antibody-negative patients with recent-onset undifferentiated arthritis. Rheumatol Int. 33(9):2309-14. 2013.

[4]. Yamanaka H, Matsuda Y, Tanaka M, Sendo W, Nakajima H, Taniguchi A, et al. Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early rheumatoid arthritis. Arthritis Rheum. 43(4):852-8. 2000.

[5]. Okada Y, Naka K, Kawamura K, Matsumoto T, Nakanishi I, Fujimoto N, et al. Localization of matrix metalloproteinase 9 (92-kilodalton gelatinase/type IV collagenase = gelatinase B) in osteoclasts: implications for bone resorption. Lab Invest. 72(3):311-22. 1995.

[6]. Ma JD, Zhou JJ, Zheng DH, Chen LF, Mo YQ, Wei XN, et al. Serum matrix metalloproteinase-3 as a noninvasive biomarker of histological synovitis for diagnosis of rheumatoid arthritis. Mediators Inflamm. 2014:179284. 2014.

[7]. Tchetverikov I, Lard LR, DeGroot J, Verzijl N, TeKoppele JM, Breedveld FC, et al. Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. Ann Rheum Dis. 62(11):1094-9. 2003.

[8]. Green MJ, Gough AK, Devlin J, Smith J, Astin P, Taylor D, et al. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. Rheumatology (Oxford). 42(1):83-8. 2003.

[9]. Tetlow LC, Woolley DE. The effects of 1 alpha,25-dihydroxyvitamin D(3) on matrix metalloproteinase and prostaglandin E(2) production by cells of the rheumatoid lesion. Arthritis Res. 1(1):63-70. 1999.

[10]. Galil SM, El-Shafey AM, Hagrass HA, Fawzy F, Sammak AE. Baseline serum level of matrix metalloproteinase-3 as a biomarker of progressive joint damage in rheumatoid arthritis patients. Int J Rheum Dis. 19(4):377-84. 2016.

[11]. Lohmander LS, Brandt KD, Mazzuca SA, Katz BP, Larsson S, Struglics A, et al. Use of the plasma stromelysin (matrix metalloproteinase 3) concentration to predict joint space narrowing in knee osteoarthritis. Arthritis Rheum. 52(10):3160-7. 2005.

[12]. Posthumus MD, Limburg PC, Westra J, van Leeuwen MA, van Rijswijk MH. Serum matrix metalloproteinase 3 levels during treatment with sulfasalazine or combination of methotrexate and sulfasalazine in patients with early rheumatoid arthritis. J Rheumatol. 29(5):883-9. 2002.

[13]. Taylor DJ, Cheung NT, Dawes PT. Increased serum proMMP-3 in inflammatory arthritides: a potential indicator of synovial inflammatory monokine activity. Ann Rheum Dis.53(11):768-72.1994.

[14]. Urata Y, Uesato R, Tanaka D, Nakamura Y, Motomura S. Treating to target matrix metalloproteinase 3 normalisation together with disease activity score below 2.6 yields better effects than each alone in rheumatoid arthritis patients: T-4 Study. Ann Rheum Dis. 71(4):534-40. 2012.

[15]. Sun S, Bay-Jensen AC, Karsdal MA, Siebuhr AS, Zheng Q, Maksymowych WP, et al. The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. BMC Musculoskelet Disord. 2012;15:93. 2012.

[16]. Ma JD, Wei XN, Zheng DH, Mo YQ, Chen LF, Zhang X, et al. Continuously elevated serum matrix metalloproteinase-3 for 3 ~ 6 months predict one-year radiographic progression in rheumatoid arthritis: a prospective cohort study. Arthritis Res Ther. 17:289. 2015.

[17]. Nagashima T, Minota S. Caution is needed when interpreting changes of matrix metalloproteinase-3

in patients with rheumatoid arthritis. Rheumatol Int. 34(7):1025-6. 2014.

[18]. Ribbens C, Martin y Porras M, Franchimont N, Kaiser MJ, Jaspar JM, Damas P, et al. Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: relationship with synovitis and steroid treatment. Ann Rheum Dis.61(2):161-6.2002.

[19]. Murphy G, Cockett MI, Stephens PE, Smith BJ, Docherty AJ. Stromelysin is an activator of procollagenase. A study with natural and recombinant enzymes. Biochem J. 248(1):265-8. 1987.

[20]. Ogata Y, Enghild JJ, Nagase H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. J Biol Chem. 267(6):3581-4. 1992.

[21]. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, et al. Matrix metalloproteinases: a review. Crit Rev Oral Biol Med. 4(2):197-250. 1993.

[22]. Wu JJ, Lark MW, Chun LE, Eyre DR. Sites of stromelysin cleavage in collagen types II, IX, X, and XI of cartilage. J Biol Chem. 266(9):5625-8. 1991.

[23]. Flannery CR, Lark MW, Sandy JD. Identification of a stromelysin cleavage site within the interglobular domain of human aggrecan. Evidence for proteolysis at this site in vivo in human articular cartilage. J Biol Chem. 267(2):1008-14. 1992.

[24]. Maksymowych WP, Landewe R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H, et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. Arthritis Rheum. 56(6):1846-53. 2007.

[25]. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci. 115(Pt 19):3719-27. 2002.

[26]. Cawston TE, Mercer E. Preferential binding of collagenase to alpha 2-macroglobulin in the presence of the tissue inhibitor of metalloproteinases. FEBS Lett. 1986;209(1):9-12.

[27]. Sottrup-Jensen L. Alpha-macroglobulins: structure, shape, and mechanism of proteinase complex formation. J Biol Chem. 264(20):11539-42. 1989.

[28]. Chen JJ, Huang JF, Du WX, Tong PJ. Expression and significance of MMP3 in synovium of knee joint at different stage in osteoarthritis patients. Asian Pac J Trop Med. 7(4):297-300. 2014.